CHARACTERIZATION AND ROLE OF POSSIBLE BIOLOGICAL MARKERS IN SPLENIC MARGINAL ZONE LYMPHOMA

IL DOTTORE
GIOVANNI FRANCO

IL COORDINATORE
PROF. CALOGERO CARUSO

IL TUTOR
PROF. SALVATRICE MANCUSO

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1. SPLENIC MARGINAL ZONE LYMPHOMA (SMZL)

1.1 Definition of SMZL

Splenic Marginal Zone Lymphoma (SMZL) is a defined low-grade B-cell lymphoma, it is recognized as a distinct entity in the WHO (World Health Organization) classification.\(^1\) SMZL is considered a B cell neoplasm composed of small lymphocytes which surround and replace the splenic white pulp germinal centres, efface the follicle mantle and merge with a peripheral (marginal) zone of larger cells including scattered transformed blasts. Both small and larger cells infiltrate the red pulp. Splenic hilar lymph nodes and bone marrow are often involved. Peripheral blood involvement is usually limited and in about half of cases the circulating neoplastic lymphocytes display a characteristic villous appearance.

SMZL is characterized by an almost exclusive involvement of the spleen and bone marrow.\(^3\)\(^-\)\(^5\)

Signs and symptoms are related to the presence of cytopenias in to peripheral blood and or to the abdominal discomfort due to a huge splenomegaly.\(^5\)\(^-\)\(^11\)

1.2 Epidemiology

Although it is considered as a rare neoplasm accounting for about 2% of all non Hodgkin’s lymphomas (NHL) it is
estimated represents for most cases of otherwise unclassifiable chronic lymphoid B-cell CD5-lymphoproliferative disorders. The peak of incidence is in the sixth decade with an equal female/male ratio.

1.3 Diagnosis of SMZL

The gold standard diagnostic assay is the study of spleen and bone marrow histology.1,12

Recently, to avoid a surgical procedure for diagnostic purposes in otherwise healthy subjects, the Splenic Lymphoma Group proposed guidelines for the diagnosis of SMZL based on the integration of bone marrow and peripheral blood picture with immunophenotypic and cytogenetic data (Table 1, Figure 1).13,14

Table 1. SMZL diagnostic and staging work-up

<table>
<thead>
<tr>
<th></th>
<th>Full blood count (FBC) with differential counts, reticulocytes, Coomb’s test and autoimmune screen (ANA, anti-DNA, AMA, anti-thyroid, rheumatoid factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Renal and liver biochemistry including calcium levels and LDH</td>
</tr>
<tr>
<td>3</td>
<td>Serum and urine Igs and B-2 microglobulin</td>
</tr>
<tr>
<td>4</td>
<td>Serology for hepatitis C (if positive, reverse transcriptase- PCR for HCV RNA in the blood) and virus genotyping, when possible</td>
</tr>
<tr>
<td>5</td>
<td>HIV serology should be investigated, with the limitations due to specific countries policies</td>
</tr>
<tr>
<td>6</td>
<td>Review of blood morphology and flow cytometry (if circulating</td>
</tr>
</tbody>
</table>
lymphoma cells)

7 BM aspirate with morphology and flow cytometry and trephine biopsy with immunohistochemistry (IHC). BM aspirate in the absence of trephine biopsy and immunohistochemistry may have a low diagnostic value.

8 Computerized tomography scan of the abdomen and chest

---

**OPTIONAL**

1 Helicobacter pylori (if the patient has gastric symptoms) and full viral screen, including hepatitis B virus, Epstein Barr Virus (EBV) and cytomegalovirus (CMV) by PCR

2 Thyroid function if anti-thyroid antibodies have been detected

3 Fluorescent in situ hybridization/cytogenetic analysis in the relevant sample involved (to exclude the diagnosis of other B-cell lymphomas in cases with uncertain diagnosis, particularly CLL or MCL in CD5 + cases and FL)

4 Cryoglobulins if HCV positive

5 On the basis of these investigations and once the diagnosis of SMZL +/- VL has been established, the patients can be stratified into

(c) Early/asymptomatic SMZL +/- VL. These comprise patients with no B-symptoms, absent or mild cytopenias, non-bulky splenomegaly, no significant (<2 cm) lymphadenopathy and no evidence of symptomatic or active hemolysis. BM infiltration may be present but with a good hemopoietic reserve

(d) Advanced/symptomatic SMZL +/- VL comprises patients with symptomatic cytopenias, progressive splenomegaly, rapidly raising lymphocyte counts and/or development of lymphadenopathy or involvement of extranodal sites.

Adapted from: Matutes et al., Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. Leukemia, 22(3):487-495, 2008
Figure 1. Schematic representation of the main steps of the SMZL diagnostic workup

Adapted from: Iannitto E, Tripodo C: How I diagnose and treat splenic lymphomas.blood-2010-2009-271437, 2010
**Blood cytology**

Blood involvement may be detected morphologically and or by flow cytometry. It is characterized by the presence of circulating lymphocytes having a round nucleus with condensed chromatin and basophilic cytoplasm with short villi, which may be unequally distributed or concentrated at one or the two poles of the cell. There is often a degree of morphological heterogeneity shown by the presence of small lymphoid cells without specific features, lymphoplasmacytoid cells, lymphocytes with nuclear clefts or medium-sized lymphoid cells with relative abundant pale cytoplasm (like monocytoïd cells). The presence of nucleolated and large cells with immature chromatin usually correlates with disease progression or even transformation to a large-cell lymphoma. The term SLVL has been widely used as an indicator of the blood manifestation of SMZL; however, in view of the possibility of technical artifact described above and the lack of agreement on the required numbers or proportion of circulating villous (VL) lymphocytes to label a case as SLVL, WHO recommendation suggests to use the term SMZL +/- VL lymphocytes. (Figure 2)
**1.5 Flow cytometry immunophenotype**

The proportion of clonal B cells varies widely from 5 to 90%. In the large majority of patients, the monoclonal B cells express moderate to strong intensity of IgM and IgD or IgM alone. In the remaining, the cells are IgG +, IgD + and rarely IgM/A + or IgA +. In the majority of patients, the cells are CD20+, CD22+, CD24+, CD27+, FMC7+ and have strong
expression of CD79b (MoAb that identifies the β chain of the B-cell receptor).

1.6 Bone Marrow Histology

Bone marrow (BM) aspirate is not sufficient for the diagnosis, and BM trephine histology is always requested for an exact diagnosis. BM involvement in patients with SMZL is invariably observed and is better detected after immunohistochemical staining.³

In the early phase of the disease, BM involvement can be slight, less than 20% of total cellularity. The pattern of infiltration is typically intrasinusoidal and with the progression of the disease, especially after splenectomy it becomes nodular.¹⁶ In advanced cases, the association of intrasinusoidal and nodular infiltration is characteristic, although not entirely specific.¹⁷ Frequently the follicles in the BM show a preserved germinal center surrounded by a rim of marginal zone cells. In a minority of cases, interstitial infiltration can occur with intrasinusoidal and/or nodular, but never alone. In the smallest sinusoids, tumor cells are typically arranged in Indian files. The cell morphology is monomorphistic. Neoplastic cells have small to medium size, round to oval nucleus with regular contour and a small rim of cytoplasm. Plasmacytoid features can be observed in a small percentage of cases. (Figure 3)
Immunohistochemical analysis shows a mature B phenotype. CD20, CD45RA, bcl2 are regularly positive, while cyclin D1, CD10 and bcl6 are negative. A minority of cases (10–15%) can be positive for CD5 and/or CD43, although they are usually negative for CD23. The neoplastic B cells are light chain restricted and a light chain restricted plasma cell component is sometimes present as well.  

The differential diagnosis includes all other small B-cell and T-cell disorders. Immunohistochemistry is fundamental to differentiate SMZL from these conditions. A main diagnostic problem arises with lymphoplasmacytic and marginal nodal lymphomas. In these cases, a lymph node biopsy or additional cytogenetic studies can help to solve the problem. Reactive conditions, such as the benign lymphoid hyperplasia associated with HCV infection, can be confused with SMZL, but almost never have intrasinusoidal component. An important exception is persistent polyclonal B lymphocytosis, which may show intrasinusoidal infiltration and thus mimic SMZL.
Figure 3. Bone marrow patterns of infiltration in SMZL


1.7 Cytogenetics in SMZL

Complex chromosomal aberrations are common, about 70-80% of patients have an abnormal karyotype. The most frequent cytogenetic aberrations are gains of 3q (20–30% of cases) and 12q (15–20% of cases), and deletion of 7q22–36, frequently at 7q32 (30–40%). The chromosomes most frequently involved are 1, 3, 6, 7, 8, 12 and 14.19-25 The highest incidence of genetic loss is found in band 7q32 although distinct regions of loss both
centromeric and telomeric to this region have also been identified. Translocations involving the Ig heavy- or light-chain loci are uncommon in SMZL. They may occur either as primary or secondary cytogenetic abnormalities. No evidence of the t(11;18) (q21;q21), t(14;18)(q32; qq21) or t(3;14)(p14;q32) associated with MALT lymphoma has been found in SMZL. Some series revealed the presence of t(11;14)(q13;q32) in few cases, and it has been suggested that the molecular breakpoint could be different from mantle cell lymphoma cases. Deletion of chromosome 17p13 occur in less than 5% of SMZL cases but mono-allelic p53 loss has been found in 17% of patients in one series. Comparative genomic hybridization has shown chromosome gains or losses in 83% of patients with a median of four abnormalities per case. In addition to the common abnormalities detected cytogenetically, gains of chromosome 5q, 12p, 20q and 9p have been identified by CGH. However, several of these abnormalities have been reported in other small B-cell lymphomas, including trisomy 3/3q in MCL and MALT lymphoma, trisomy 12 in CLL and MALT lymphoma, trisomy 18 in FL and MALT lymphoma and del7q in CLL, MCL and LPL.

Another recent retrospective collaborative study analysed 330 patients affected by SMZL. In 239 patients (72%) is reported an aberrant karyotype, 53% were complex and 29% had a single aberration. The predominant aberrations were
gains of 3/3q (N=60/239; 25%) and 12q (N=8/21; 21%),
deletions of 7q (N=94/239; 39%) and 6q (N028/239; 11.7%).
In this study some parameters affected the prognosis but
only in univariate analyses, in multivariate analyses
cytogenetic aberrations did not retain prognostic value.
Therefore, it is important that the cytogenetic findings be
correlated with the clinical, morphological and
immunophenotypic features before a diagnosis of SMZL is
made.

1.8 IGVH mutational status in SMZL

Several studies has evaluated the role of the mutational
status of the IGVH (Immunoglobulin Heavy Chain Variable
region genes).\textsuperscript{28-39}
In chronic lymphocytic leukemia the evaluation of the IGVH
mutational status has reported as an important prognostic
factor, particularly the status of “unmutated” is associated
with a worse prognosis, on the other hand the mutational
status “mutated”, typical of that B-cells that have
encountered the antigen, is associated with a better
prognosis.\textsuperscript{40}
Table 2 summarizes the results of these studies on IGVH
mutational status in patients with SMZL. In literature the
percentage of “mutated” cases ranges from 50 to 90-100%,
and in some reports an attempt to correlate these results
with prognosis even in SMZL was made. Nonetheless case
series are very poor, they varies from 5 to 50-70 patients at all. Further analyses are requested to better define the role of the IGVH mutational status in SMZL.

*Table 2. Studies on IGVH mutation status in SMZL*

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>N. of patients</th>
<th>% of mutated cases</th>
<th>Prognostic correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marasca²⁸</td>
<td>2001</td>
<td>10</td>
<td>90</td>
<td>n.a.</td>
</tr>
<tr>
<td>Wang²⁹</td>
<td>2002</td>
<td>5</td>
<td>100</td>
<td>n.a.</td>
</tr>
<tr>
<td>Bahler³⁰</td>
<td>2002</td>
<td>8</td>
<td>50</td>
<td>n.a.</td>
</tr>
<tr>
<td>Algaras³¹</td>
<td>2002</td>
<td>35</td>
<td>50</td>
<td>Yes</td>
</tr>
<tr>
<td>Tierens³²</td>
<td>2003</td>
<td>23</td>
<td>70</td>
<td>No</td>
</tr>
<tr>
<td>Stamatopoulos³³</td>
<td>2004</td>
<td>43</td>
<td>59</td>
<td>n.a.</td>
</tr>
<tr>
<td>Ruiz-Ballestreros³⁴</td>
<td>2005</td>
<td>44</td>
<td>59</td>
<td>n.a.</td>
</tr>
<tr>
<td>Traverse-Glehen³⁵</td>
<td>2005</td>
<td>49</td>
<td>69</td>
<td>n.a.</td>
</tr>
<tr>
<td>Papadaky³⁶</td>
<td>2007</td>
<td>42</td>
<td>62</td>
<td>n.a.</td>
</tr>
<tr>
<td>Arcaini³⁷</td>
<td>2009</td>
<td>55</td>
<td>75</td>
<td>Yes</td>
</tr>
<tr>
<td>Kalpadakis³⁸</td>
<td>2009</td>
<td>22</td>
<td>59</td>
<td>No</td>
</tr>
</tbody>
</table>

1.9 *Prognostic factors in SMZL, and identification of patients with a worst prognosis*

SMZL usually runs an indolent clinical course, but some patients manifest with a more aggressive disease. Clinical and cytogenetic parameters with prognostic value are not well established. Only a few studies, including small numbers of cases and analysing selected parameters, have been reported.
SMZL is a neoplasm of the elderly with a median age of 70 years, the median survival exceeds ten years and most patients can be managed for many years with a watchful waiting policy. Yet in about twenty-five per cent of cases the disease follows a more aggressive course and most of the patients die of lymphoma progression within 3-4 years. Some presenting features on diagnosis have been shown to have a significant prognostic value such as extranodal involvement, high lymphocyte count, lymphonodal involvement, anemia and thrombocytopenia. When patients develop during one or more of these conditions the course of the disease is towards disease progression, and consequently they have a worse prognosis.\textsuperscript{6-8,11}

The Intergruppo Italiano Linfomi (IIL) has proposed a clinical score based on three parameters\textsuperscript{41}: anemia, elevated LDH values and hypoalbuminemia (Table 3,4; Figure 4).
Table 3. Clinical parameters influencing Overall-Survival (OS) and Cause-Specific Survival (CSS) in SMZL and Intergruppo Italiano Linfomi (IIL) prognostic score for SMZL.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Overall Survival</th>
<th>Cause-Specific Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>P</td>
</tr>
<tr>
<td>Hemoglobin level less than 12 g/dl</td>
<td>2.7</td>
<td>.005</td>
</tr>
<tr>
<td>LDH level higher than normal</td>
<td>2.2</td>
<td>.008</td>
</tr>
<tr>
<td>Albumin level less than 3.5 g/dl</td>
<td>3.2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Score**

- **Low risk**: None of the adverse factor
- **Intermediate risk**: One adverse factor
- **High risk**: Two or three adverse factors

Table 4. Overall-Survival (OS) and Cause-Specific Survival (CSS), deaths, mortality rates of patients with SMZL grouped in the three categories of the Intergruppo Italiano Linfomi (IIL) prognostic model

<table>
<thead>
<tr>
<th></th>
<th>Low risk</th>
<th>Intermediate risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>5y OS, % (95% CI)</td>
<td>83 (71-94)</td>
<td>72 (58-85)</td>
<td>56 (37-74)</td>
</tr>
<tr>
<td>10y OS, % (95% CI)</td>
<td>83 (71-94)</td>
<td>72 (58-85)</td>
<td>NR</td>
</tr>
<tr>
<td>5y CSS, % (95% CI)</td>
<td>88 (77-98)</td>
<td>73 (58-88)</td>
<td>50 (33-67)</td>
</tr>
<tr>
<td>10y CSS, % (95% CI)</td>
<td>88 (77-98)</td>
<td>73 (58-88)</td>
<td>NR</td>
</tr>
<tr>
<td>Deaths, %</td>
<td>20</td>
<td>31</td>
<td>49</td>
</tr>
<tr>
<td>Cause-specific deaths, %</td>
<td>16</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>Mortality rate, x 1000</td>
<td>27</td>
<td>60</td>
<td>201</td>
</tr>
<tr>
<td>person-years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cause-specific mortality</td>
<td>20</td>
<td>47</td>
<td>174</td>
</tr>
<tr>
<td>rate, x 1000 person-years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients showing two or more of the above adverse prognostic factors have a median life expectancy of less than five years.

For the time being, there is not a prospectively validated therapeutic approach for SMZL and splenectomy has been considered to be the therapeutic approach most effective in patients complaining signs and symptoms secondary to hyperpslenism. However, though almost all patients achieve a clinical response after splenectomy they virtually always relapse or progress after this procedure. Furthermore, most
of patients affected by SMZL are elderly and show comorbidities that classify them as poor surgical risk.\textsuperscript{42-44}

A recent analysis\textsuperscript{45} founds a role of the microenvironment of the bone marrow in determining the prognosis of the SMZL patients. Authors investigate the effect of stroma-intrinsic features on SMZL disease progression by focusing on the microenvironment of the bone marrow, which represents an elective disease localization endorsing diagnostic and prognostic relevance. The study shows that the quality of the BM stromal meshwork of SMZL infiltrates correlates with time to progression. In particular the unfavorable prognostic influence of dense CD40 expression by BM stromal cells, which involves the contribution of CD40 ligand (CD40L)-expressing bystander mast cells infiltrating SMZL BM aggregates. The CD40/CD40L-assisted crosstalk between mesenchymal stromal cells and mast cells populating the SMZL microenvironment finds correlation in p53(-/-) mice developing SMZL and contributes to the engendering of detrimental proinflammatory conditions. This study highlights a dynamic interaction, playing between non-neoplastic elements within the SMZL niche, toward disease progression.

\textbf{1.10 Therapeutic approach to SMZL}

For patients who show a dissemination of the disease to lymph nodes or other extranodal sites presence of adverse
prognostic factors or peripheral cytopenias a systemic treatment may be appropriate (Figure 1, Table 5).

Table 5. Situations requiring starting a systemic treatment

<table>
<thead>
<tr>
<th>At least one of the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>Symptomatic SMZL in not splenectomized patients</strong></td>
</tr>
<tr>
<td>a) <strong>Bulky</strong> (arbitrarily defined as ≥ 6 cm below left costal margin) or progressive or painful splenomegaly, without enlarged lymphoadenopathy with or without cytopenia, not eligible for splenectomy or not willing splenectomy.</td>
</tr>
<tr>
<td>b) <strong>One of the following symptomatic/progressive cytopenias:</strong> Hb &lt; 10 g/dL, or Platelets &lt; 80,000/mm³, or neutropenia &lt; 1,000/mm³, whatever the reason (autoimmune or hypersplenism or bone marrow infiltration) not eligible for splenectomy or not willing splenectomy.</td>
</tr>
<tr>
<td>c) <strong>SMZL with enlarged lymphoadenopathy or involvement of extranodal sites, with or without cytopenia.</strong></td>
</tr>
<tr>
<td>2) Symptomatic disease in SMZL splenectomised patients with rapidly raising lymphocyte counts, development of lymphadenopathy or involvement of extranodal sites.</td>
</tr>
<tr>
<td>3) <strong>SMZL with concomitant hepatitis C infection who have not responded to or are relapsed after Interferon and/or Ribavirin</strong></td>
</tr>
</tbody>
</table>

Retrospective studies have indicated that alkylating-agents monotherapy do not produce any clinical benefit while
purine analogs (i.e. fludarabine, cladribine, and pentostatine) achieved very high response rates in both naive and pre-treated patients.\textsuperscript{46-48}

Introduction of the anti-CD20 humanized antibody rituximab, as single agent or in combination with other chemotherapy agents has been reported to be very effective in producing a rapid clearance of neoplastic cells. Treatment of such patients with rituximab both alone or in combination with chemotherapy has shown remarkable responses.\textsuperscript{49-57}

1.10.1 \textit{R-COMP (Rituximab, Cyclophosphamide, Oncovin, Myocet, Prednisone) in the treatment of Splenic Marginal Zone Lymphomas}

In 2005 the Intergruppo Italiano Linfomi started a prospective phase II trial to verify the efficacy of rituximab in combination with COMP (Cyclophosphamide, Oncovin, Myocet, Prednisone) chemotherapy, as front-line therapy for SMZL patients, in terms of response, survival, and safety. To be included in the trial, patients should had a diagnosis of SMZL supported by spleen histology or by a combination of bone marrow and peripheral blood morphologic and immunophenotypic data. Patients should also be untreated for lymphoma and have active disease defined by the presence of at least one of the following: Hemoglobin < 10g/dl; Platelets < 100,000/mmc, symptomatic splenomegaly, elevated LDH serum level, presence of B
symptoms (fever, weight loss more than 10% of total body weight, night sweats), extrasplenic disease. The preliminary results of this study have been presented at 2007 ASH meeting. The most important findings were an ORR of 100% with 63% of CR. The results have been recently update, confirming on 63 patients an 85% ORR with 63% CR rate, and a 82% PFS at three years. However, the toxicity profile showed a 24% haematological toxicity WHO 3-4 and one patients died of therapy-related cardiac failure while in CR.

1.10.2 Bendamustine in the treatment of indolent and marginal zone lymphomas

Preclinical studies demonstrate the efficacy of bendamustine against cancer cells resistant to other alkylating agents. Alkylator-resistant human B-CLL cells are less resistant to bendamustine and there is clinical evidence that bendamustine preserves activity when other alkylators fail.

In these analyses the overall remission rate was 56%, with a median duration of remission of 42.7 months. Several studies of bendamustine based therapy in patients with relapsed and refractory low-grade lymphomas report overall remission rates from 48% to 97%.

Therefore, both preclinical and clinical data suggest that bendamustine may have properties distinct from other alkylating agents.
Several experiences have been published related to the use of bendamustine as a single or combined agent in low-grade lymphomas. As a single agent, bendamustine was administered to 52 evaluable patients with relapsed or refractory low-grade lymphomas at a dose of 120 mg/m² on the first 2 days of a 21-day cycle. The median age of the patients was 63 years (range, 36-82 years), and all patients had been previously exposed to alkylating agents. The overall response rate (ORR) was 73%, and 11% achieved complete remission (CR). The regimen appeared to be remarkably well tolerated, and only 3 patients developed grade 3 or 4 toxicity, both grade 3 neutropenia. This study and similar studies conducted in Europe suggest activity of bendamustine against low grade lymphoma in the absence of debilitating or unmanageable toxicity. To support confirmation of these preliminary observations, another study was organized to test the safety and efficacy of bendamustine in rituximab refractory patients with indolent and transformed non-Hodgkin lymphoma. Patients enrolled on this study were defined as rituximab-refractory if they failed to respond or progressed within 6 months of previous treatment with rituximab. This multicenter trial enrolled 77 patients, and 76 patients were treated with bendamustine 120 mg/m² on the first 2 consecutive days of a 21-day cycle. Nineteen patients required dose reductions. The incidence of grade 3-4 neutropenia was 54% and of grade 3-4 thrombocytopenia was 25%. Non-hematologic toxicities were common, but
generally mild with grade 1-2 nausea in 68% and grade 1-2 fatigue in 42%. No patient developed alopecia or mucositis. Although these patients were frequently relapsed from combination chemotherapy regimens, and 20% had transformed disease, the ORR was 77%, and 34% achieved CR. Interestingly, the ORR in patients with alkylator-resistant disease was 61%, and it was 62% in the 8 patients with fludarabine-refractory disease. For all patients, the median progression-free survival (PFS) was 7.1 months. The results of this study confirm earlier findings and clearly determine bendamustine as a safe and effective treatment for relapsed and refractory low-grade lymphomas. Although remission rates were high, however, the duration of remission was relatively short. Thus, bendamustine has been combined with other active agents to determine whether results could be improved.

Therefore, rituximab has been combined with bendamustine (BR) for the treatment of low-grade lymphoma in some recent clinical trials. A German study accrued 63 patients with relapsed or refractory low-grade non-Hodgkin lymphoma for treatment with BR. Rituximab 375 mg/m² was administered on Day 1, and bendamustine was administered at a dose of 90 mg/m² on Days 2 and 3 of a 28-day treatment cycle. Rituximab was also infused 1 week before the first treatment cycle and 4 weeks after the fourth and final treatment cycle. The median age of the patients was 64 years (range, 40 to 81 years). The beta-2 microglobulin level was
>2 mg/dL in 48%, and the lactate dehydrogenase level was
>240 U/L in 24% of assessable patients. Toxicity was graded
according to World Health Organization (WHO) guidelines
and was limited to myelosuppression (16% grade 3-4
leukopenia) and nausea (102 of 136 treatment cycles
complicated by grade 1 nausea). Hematopoietic growth
factors (HGF) were allowed but none of the patients used
HGF. The ORR rate to BR on this study was 90%, and 60%
achieved a CR. Patients with relapsed mantle cell lymphoma
had an ORR of 75%, and 50% achieved CR. These remissions
were also reasonably durable, with a median progression-
free survival of 24 months. The median PFS in patients with
mantle cell lymphoma was 18 months. The remarkable
results of the German study of BR prompted a confirmatory
study in North America. The treatment regimen was identical
to the German study, but toxicity was graded according to the
National Cancer Institute’s Common Terminology Criteria
(NCI-CTC). Sixty-seven patients with relapsed or refractory
low-grade non-Hodgkin lymphoma were enrolled in this trial,
and 66 patients were treated with BR.67 This population of
patients had a median age of 60 years (range, 40-84 years)
and was similar to the German cohort. The efficacy results of
the North American Trial were also very similar to those of
the German trial. Both studies demonstrate excellent activity
against low-grade lymphomas across multiple histologic
subtypes. The two studies differed in their assessment and
reporting of toxicity, leading to apparent discrepancies in
their respective toxicity results. In the German study, toxicity was reported as a function of events per treatment cycle, whereas in the North American study toxicity was reported as a function of study participants. Grade 3-4 leukopenia was reported in 16% of treatment cycles in the former study, and in 30% of study participants in the latter study. The discrepancy is more than just in the way toxicity was reported. Therefore, the toxicity in the 2 trials is not as easily compared as the response rates are. Nonetheless, severe adverse events were uncommon in both studies.

Considering this treatment option bendamustine is neither a new drug, nor a biologically targeted agent. Yet it has unique activity against lymphoproliferative disorders, and its favorable side-effect profile makes it amenable for use in combination with other agents. Further investigations are needed in the specifically field of SMZL treated with Bendamustine based regimen. Recently use of Bendamustine in association with Rituximab was allowed in the treatment of SMZL even in first line therapy after a large study that showed a non-inferiority efficacy of R-Bendamustin versus standard therapy, that is R-CHOP.71

In this prospective large trial multicentric were randomised 274 patients to bendamustine plus rituximab treatment versus 275 to R-CHOP treatment, affected by indolent and mantle cell lymphomas untreated, in these series were comprised also marginal zone lymphomas. Bendamustine was used at the dosage of 90mg/m² every 28 days.
At median follow-up of 45 months median progression-free survival was significantly longer in the bendamustine plus rituximab group than in the R-CHOP group (69.5 months vs 31.2 months; HR 0.58, 95% CI 0.44-0.74; p<0.0001). Bendamustine plus rituximab was better tolerated than R-CHOP, with lower rates of alopecia (0 patients vs 245 - 100%) of 245 patients who received ≥3 cycles; p<0.0001), haematological toxicity 30% vs 68%; p<0.0001, infections 37% vs 50%; p=0.0025, peripheral neuropathy 7% vs 29%; p<0.0001, and stomatitis 6% vs 19%; p<0.0001). Erythematous skin reactions were more common in patients in the bendamustine plus rituximab group than in those in the R-CHOP group 16% vs 23 9%; p=0.024). Authors concluded that in patients with previously untreated indolent lymphoma, bendamustine plus rituximab can be considered as a preferred first-line treatment approach to R-CHOP because of increased progression-free survival and fewer toxic effects (Figure 5).
Figure 5. Progression Free Survival (PFS) in patients affected by marginal zone lymphomas treated with Bendamustine + Rituximab (B-R) versus R-CHOP.

2. AIM OF THE STUDY

SMZL is a neoplasm with an indolent course in about two third of patients. It is characterized by progressive splenomegaly and consequent anemia, neutropenia and or decreasing in platelets count, sometimes accompanied by autoimmune disorders. The bone marrow involvement, that is present in almost all cases of SMZL, and the recurrent presence of villous lymphocytes in the peripheral blood, makes SMZL a very interesting biological model to study. In fact SMZL, even if considered as a lymphoma, can be studied as a “leukemic” model of B cell neoplasm for its virtually always involvement of bone marrow, consequently biological studies can be performed on several tissues (blood, spleen, bone marrow). The aim of this study is to characterize the possible role of some biological markers in order to identify those patients with a more aggressive course of disease. In this study attention was focused on patients with an “active disease”, namely those patients that requiring starting a systemic treatment, to analyse if some biological differences are present in these patients on the contrary of what described yet in literature where patients at diagnosis are almost evaluated.
3. MATERIALS AND METHODS

3.1 *Cases selection and inclusion criteria*

Patients that received diagnosis of SMZL were enrolled in this study according to the following inclusion criteria:

- Initial diagnosis of CD20+ Splenic Marginal Zone Lymphoma (According to WHO 2008 classification of Lymphoma) morphology confirmed by histology, cytology, and immunophenotype or according to the recommendation of the Splenic Lymphoma Group for non splenectomized patient.
  - If patients not splenectomised: diagnosis on bone marrow biopsy and blood (cytology and immunophenotype - chromosomal abnormalities)
  - If patients splenectomised diagnosis on spleen, bone marrow biopsy (histology and immunophenotype), and blood (cytology and immunophenotype - chromosomal abnormalities)
- No previous treatment with immunotherapy or chemotherapy or radiotherapy unless pretreatment by monocorticotherapy.
- Patients requiring a treatment with at least one of the following situation:
  1. Symptomatic SMZL in not splenectomized patients
     a) Bulky (arbitrarily defined as ≥ 6 cm below left costal margin) or progressive or painful
spleomegaly, without enlarged lymphohadenopathy with or without cytopenia, not eligible for splenectomy or not willing splenectomy

b) one of the following symptomatic/progressive cytopenias: Hb < 10 g/dL, or Plat < 80,000/mm³, or neutropenia < 1,000/mm³, whatever the reason (autoimmune or hypersplenism or bone marrow infiltration) not eligible for splenectomy or not willing splenectomy.

c) SMZL with enlarged lymphohadenopathy or involvement of extranodal sites, with or without cytopenia.

2. Symptomatic disease in SMZL splenectomised patients with rapidly raising lymphocyte counts, development of lymphadenopathy or involvement of extranodal sites.

3. SMZL with concomitant hepatitis C infection who have not responded to or are relapsed after Interferon and/or Ribavirin

3.2 Molecular analysis

Flow-cytometry immunophenotype, IgVH gene rearrangement analyses and Cytogenetic assessment are performed on the peripheral blood and the bone marrow aspirate at diagnosis on Heparined or EDTA peripheral blood
(20 ml) and heparined or EDTA bone marrow aspirate (5-10 ml)

3.2.1 *Immunoglobulin heavy-chain variable-region (IgVH)* gene mutation analysis

The detection of IgVH gene rearrangements (B cell clonality assay) is performed by fluorochrome labeled PCR as described in the protocol BIOMED 2 PCR\textsuperscript{72}. The fluorochrome labeled PCR products will be analyzed by GeneScanning analysis using ABI prism 3100 Avant Genetic Analyser. GeneScanning analysis of IgVH rearrangements have been developed for B cell clonality studies with a sensitive of clonal populations of >1%, (not for detection of subclones <1%). Immunoglobulin heavy-chain variable-region (IGVH) gene mutation analysis is performed as previously described\textsuperscript{73}. Briefly, clonal IgVH rearrangements will be amplified from genomic DNA using 5’ primers corresponding to the VH leaders or VH framework 1 region and 3’ primers corresponding to the JH genes. PCR products will be sequenced directly by Sanger method using ABI prism 3100 Avant Genetic Analyser. Sequence analysis including identification of the closest germline gene counterpart, distribution and characteristics of somatic mutations will be performed using dedicated bioinformatic tools (IMGT V-QUEST, IgBlast).
3.2.2 Flow cytometry

Immunophenotype analysis by flow cytometry is performed on fresh Mononuclear Cells (MNC) using the following monoclonal antibodies (moAb): CD3+, CD4, CD8, CD19, CD20, CD5, CD10, CD23, CD11c, FMC7, CD103, CD25, Slg, SlgM, SlgG, SlgD, k, λ, CD22, CD43, CD103, CD25, CD123. Four color stainings will be performed using moAb conjugated with four different fluorochromes and using FACSlibur cytometer (BD biosciences). The acquired data will be analyzed by Cell Quest software.

3.2.3 Chromosomal abnormalities

Analysis of chromosomal abnormalities will be performed from DNA extracted from MNC from peripheral blood or bone marrow aspirates (only in the case of peripheral blood without neoplastic cells): +3; del7; +12; +18.

3.2.4 Immunohistochemistry

Bone marrow biopsy samples were fixed in 10% neutral buffered formalin, decalcified in EDTA, and paraffin embedded. Four-micrometers-thick sections were deparaffinized in xylene and rehydrated to water prior to microwave antigen retrieval in c Tris-HCl/EDTA pH 9.0 buffer (Dako Cytomation, Denmark) and PBS washing. After
neutralization of the endogenous peroxidase with 3% H2O2 for 10 minutes, the sections were incubated with protein block (Novocastra Leica Biosystems, Germany) for 10 min before undergoing incubation with the primary antibodies. After counterstaining with hematoxylin (Novocastra Leica Biosystems), the sections were analysed under a Leica DMD108 digital microscope (Leica Microsystems, Germany).
4. RESULTS

Thirty-seven consecutive cases of SMZL were enrolled in this study. Table 6 and Figure 6 summarize the main clinical features of these patients. The median age at enrolment is 67 (with a range of 34-78). Twenty two patients were male (59.5%) while 15 (40.5%) were females, with a male to female ratio of M:F=1.5:1. Data on risk stratification according to IIL\textsuperscript{41} prognostic score was available in 23 cases. Four (17%) patients have a high risk score, 14 patients (61%) have an intermediate risk score and 5 patients (22%) have a low risk score.

The median haemoglobin value is 12 g/dl (3-16.1), the median white blood cell count (WBC) is $11 \times 10^9$/L (3-121.1), median neutrophil count is $3.2 \times 10^9$/L (1.4-7.8), median lymphocyte count is $8.4 \times 10^9$/L (0.9-117.5), the median albumin serum level is 3.9 g/dl (3.2-5.2) and the median lactate dehydrogenase (LDH) serum level is 356 UI/L (77-822).

By flow cytometry percentage of lymphoid infiltrate in bone marrow was measured, median infiltration is of $7.1 \times 10^3$ mm$^3$ (Range 1.5-38.1). Also percentage of infiltrating clonal cells in the bone marrow was performed, there is a 65.5% (Range 15-90) presence of clonal cells.

Lymphocyte count assessed in peripheral blood by flow cytometry reveals a median of $8.5 \times 10^3$ mm$^3$ (Range 1-141).
The percentage of clonal circulating cells in peripheral blood was studied and the median is of 68% (Range 10-96).

*Table 6. Main clinical and laboratory findings in patients of the study*

<table>
<thead>
<tr>
<th>Total of cases N. 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>Hemoglobin (g/dL); Median (Range)</td>
</tr>
<tr>
<td>WBC (x10⁹/L); Median (Range)</td>
</tr>
<tr>
<td>Neutrophils (x10⁹/L); Median (Range)</td>
</tr>
<tr>
<td>Lymphocyte (x10⁹/L); Median (Range)</td>
</tr>
<tr>
<td>Platelets (x10⁹/L); Median (Range)</td>
</tr>
<tr>
<td>Albumin serum level (g/dl); Median (Range)</td>
</tr>
<tr>
<td>LDH serum level (U/L); Median (Range)</td>
</tr>
<tr>
<td>Lymphocyte infiltrate in Bone Marrow (x10³mm³); Median (Range)</td>
</tr>
<tr>
<td>Percentage of clonal cells in Bone Marrow; % (Range)</td>
</tr>
<tr>
<td>Lymphocyte in peripheral blood revealed by flow cytometry (x10³mm³); Median (Range)</td>
</tr>
<tr>
<td>Percentage of clonal cells in Peripheral Blood; % (Range)</td>
</tr>
</tbody>
</table>
Flow cytometry analysis, available on 34 patients at diagnosis or on blood marrow or peripheral blood, revealed the following immunophenotype: CD20+ CD22+ FMC7+ CD11c+/- CD5-/+ CD25-/+ CD23-/+ sIgM+/- sIgD +/- sIg +/. Figure 7 shows the percentage of positivity of single CD used in flow analyses. All cases (34; 100%) are positive for CD20 and CD22, while CD5 is positive in 7 patients (21%). CD25 and CD23 are positive respectively in 11 and 6 cases (32% and 18%). FMC7 is positive in almost all patients, 31 (91%) and CD11c is positive in 24 patients (71%). The surface Ig analyses shows a IgD positivity in 22 patients (65%) while 19 cases (56%) are positive for the IgM sIg.
Figure 7. Flow cytometry immunophenotype of the SMZL patients.

![Flow Cytometry Bar Chart]

Numbers in bars indicate the percentage of positive (red) or negative (blue) cases.

Figure 8 and Table 7 summarized the main histopathological features. Immunohistochemistry studies show the following immunophenotype: CD20+ (100%; 36 cases) CD5- (80%; 20/24) IgM+ (58%; 14/24) IgD+ (81%; 17/21).

Regarding the patterns of bone marrow infiltration by SMZL aggregates, a nodular pattern is present in 23 patients (67.6%), an interstitial or intrasinusoidal pattern is detectable in respectively 33 (97.1%) and 32 (94.1%) patients. Notably is rare to find a single pattern of bone marrow infiltration in these patients, whereas the majority of cases (100% in this case series) have a mixed pattern.
comprising two or more of the characteristic pictures of infiltration of SMZL.

*Figure 8. Immunophenotype at Immunohistochemical analysis*

Numbers in bars indicate the percentage of positive (red) or negative (blue) cases.
Table 7. Histopathological findings of the patients

<table>
<thead>
<tr>
<th>Patterns of bone marrow infiltration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(N=34; 100% mixed)</td>
<td></td>
</tr>
<tr>
<td>Nodular</td>
<td>23 (67.6%)</td>
</tr>
<tr>
<td>Interstitial</td>
<td>33 (97.1%)</td>
</tr>
<tr>
<td>Intrasinusoidal</td>
<td>32 (94.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Villous Lymphocytes in peripheral blood</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>30 (83.3%)</td>
</tr>
<tr>
<td>No</td>
<td>6 (16.7%)</td>
</tr>
</tbody>
</table>

| Percentage of villous lymphocytes; Median (Range)     | 16.5% (1-80) |
| Percentage of bone marrow infiltration; Median (Range)| 45% (15-90)  |

The study of the IgVH mutational status reveals that all the patients where this analysis is available (27 cases) have a “mutated” status. Figure 9 shows all the mutations encountered in this case series and the more frequent subgroups, Table 8 summarized the subgroups of IgVH mutations. The subgroups VH4, VH3 and VH1 are the more represented with respectively 10 (37%), 8 (30%) and 7 (26%) cases, only 2 (7%) cases are in the VH5 subgroup. On the other hand regarding the JH subgroups the data are available in 26 cases. The more representative subgroups are the JH4 and JH6 with 11 (42%) and 9 (35%) patients respectively, followed by the JH3 and JH5 subgroups with 4 (15%) and 2 (8%) of patients respectively.
Figure 9. IgVH mutations of the case series

Table 8. Subgroups of IgVH mutations

<table>
<thead>
<tr>
<th>VH1</th>
<th>VH3</th>
<th>VH4</th>
<th>VH5</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (26%)</td>
<td>8 (30%)</td>
<td>10 (37%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>JH1</td>
<td>JH2</td>
<td>JH3</td>
<td>JH4</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>4 (15%)</td>
<td>11 (42%)</td>
</tr>
</tbody>
</table>

In this study the most frequently used individual IgVH genes were IGVH 1-02 6/27 (22%), IGVH 4-34 5/27 (18.5%), IGVH 3-07 3/27 (11%) and IGVH 4-59 2/27 (7%) and IGVH 5-51 2/27 (7%).
Cytogenetic assays available on 32 patients reveals that 20 patients (62.5%) have a normal karyotype, while 12 patients (37.5%) show cytogenetic aberration, that are summarized in Figure 10 and Table 9. Deletion of chromosome 7 is present in 3 (25%) patients as single aberration, +3 is present in 3 patients (25%) as single aberration, while 3 patients (25%) show +3 and +18. One patient (8.3%) per group has a +3; del7; +12, a +3; del7 and a -18.

*Figure 10. Cytogenetic aberrations of the case series*
Table 9. *Cytogenetic aberrations of the case series*

<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>20 (62.5%)</td>
</tr>
<tr>
<td>Cytogenetic aberrations</td>
<td>12 (37.5%)</td>
</tr>
</tbody>
</table>

*Frequency of cytogenetic aberrations alone or in association with other anomalies*

<table>
<thead>
<tr>
<th>Aberration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Del7</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>+3</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>+18</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>+12</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Others</td>
<td>1 (3%)</td>
</tr>
</tbody>
</table>
5. DISCUSSION

Indolent lymphomas, such as SMZL, have an indolent course but some patients experience a more rapid disease or a worse prognosis. To identify these patients is an effort that frequently has been made in literature. In other B cell neoplasms, such as CLL or follicular lymphoma have been identified some prognostic model based on both clinical and laboratory findings. More recently researchers focused their attention on biological aspects of these diseases. To characterize any biological aspects in B cell neoplasms is extremely important, because biological features can be often evaluated since the time of diagnosis, and could revealed those patients that will be undergo to an aggressive disease, and consequently to a more appropriate helpful approach. In this light splenic marginal zone lymphoma represents an excellent biological model to study. This neoplasm, even if included in the family of lymphomas, always involved the bone marrow, and therefore the peripheral blood. So SMZL embodies a model of “leukemic” B cell neoplasm with great possibilities to be studied. Different tissues can be used in this lymphoma, such as bone marrow, spleen and circulating lymphocytes to study model of lymphoma-genesis or possible biological mechanisms that distinguish a patient with a worst prognosis than others.

To study these subgroups of patients this study focused its attention to patients that requiring a starting systemic
treatment, namely intravenous chemotherapy. These patients have a disease considered as “active” or “progressive” that usually follows a period of watchful waiting were any therapeutic approach has been started. Notably that study of neoplasms with long survivals, such as SMZL, usually more than five years, is extremely difficult for question of time and only “progressive” disease is possible to evaluate. Progressive disease is considered a symptomatic disease, due to organ enlargement, splenomegaly, or peripheral cytopenia, usually anemia or thrombocytopenia. Analysing these patients and comparing these results with those present in literature, where habitually patients at diagnosis are considered, was the aim of the present study. A first consideration regards the presence of villous lymphocytes, the more extensive study of literature considering this aspect reported a prevalence of 47% of patients with circulating villous lymphocytes, while in our case series we found a statistically significant higher presence of circulating villous lymphocytes 83% (p < .001 – Figure 11).
Figure 11. Differences in presence of villous lymphocytes between this study and literature

Regarding the immunophenotype revealed by flow cytometry in this study SMZL phenotype was the following: CD20+ CD22+ FMC7+ CD11c+/- CD5-/+ CD25-/+ CD23-/+ sIgM/- sIgD-/-, whereas “+” means a positivity of more than 90% of cases and “+” or “-“ before the “/” indicates the more frequent condition. No particular differences seems to be highlighted in terms of types of CD, but comparing patients of this study with the most extensive case series of the literature regarding SMZL and flow cytometry, can be noted that a slight higher presence of CD11c positive clones (71% vs 47%
- \( p = .03 \) and a tendency to a lower presence of CD23+ patients, even if not statistically significant \( 18\% \) vs \( 31\% \) \( p=0.1; \) Figure 12).

**Figure 12.** Differences in immunophenotype revealed by flow-cytometry between this study and literature.

Cytogenetic aberrations found in this study revealed that 20 patients (62.5\%) have a normal karyotype while only 12 patients (37.5\%). Considering this result and comparing with a recent very extensive study of cytogenetic features in SMZL cases comprising 330 patients there is a significant difference (\( p=0.0002 \)). In that study 72\% of patients have any cytogenetic aberration and normal karyotype was seen only
in 28% of patients. Considering this aspect it is unlikely that patients with an active or progressive disease have more frequently a normal karyotype, such as in this study, so cytogenetic features in SMZL are not still completely cleared and further investigations are needed to better define their role in this B cell neoplasm.

Considering the IgVH mutational status in SMZL its role appears controversial than in CLL for example. Several studies\textsuperscript{28-38} have evaluated the IgVH mutational status in SMZL and its correlation with prognosis but this association is not still clear. In this study all the patients (100%) showed a “mutated” status considering IgVH genes. If patients with an active disease could be considered with a worst prognosis then a possible role for the IgVH mutational status can be considered, also as an event that could occur when the disease gain an “activated” status. In literature the percentage of cases with SMZL varies from 50 to 90% of cases, only one study had a 100% of mutation but with a very low number of patients, and only two studies correlated this condition with prognosis\textsuperscript{31,37}. Regarding subgroups of IgVH mutations involved in SMZL patients in this study and compared with literature non-significant differences are been noted. The distribution of the subgroups seems to resemble in the different case series, with a majority of cases in the VH1, VH3 and VH4 subgroups and in the JH4 and JH6 subgroups (Figure 13).
In this study the most frequently used individual IgVH genes are the same reported in literature considering the most
frequent such as IGVH 1-02, IGVH 4-34 and IGVH 3-07, while only one case of IGVH 3-30 and IGVH 4-61 were found in this study, this two individual gene are reported in literature as frequently involved in SMZL. Notably that in the patients of this study the frequency of IGVH 4-59 is present in 2 (7%) of patients and IGVH 5-51 in other 2 (7%) of patients. This two genes have a slight higher frequency in this study and when they are added with other mutations known in literature is possible to reach a 73.5% of frequent IGVH genes involved in SMZL (Table 9)

Table 9. Frequent IGVH mutations founded in this study compared with literature.

<table>
<thead>
<tr>
<th>Frequent IGVH genes involved in SMZL</th>
<th>N. of cases in this study (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH 1-02</td>
<td>6/27 (22%)</td>
</tr>
<tr>
<td>VH 4-34</td>
<td>5/27 (19)</td>
</tr>
<tr>
<td>VH 3-07</td>
<td>3/27 (11%)</td>
</tr>
<tr>
<td>VH 3-30</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>VH 4-61</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>VH 3-23</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>VH 4-59</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>VH5-51</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>Total</td>
<td>20/27 (74%)</td>
</tr>
</tbody>
</table>

Considering these results it can be assumed a role for biological features even in SMZL like reported in several
other B cell neoplasms. What are exactly the biologic features to be investigated is not still clear and to correlate these aspects with a prognostic role are necessary further investigation in prospective study with a long time period of observation of patients considering the long survivals of these patients.
6. REFERENCES


15. Matutes E, Owusu-Ankomah K, Morilla R, et al. The immunological profile of B-cell disorders and proposal of a


