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ORIGINAL PAPER

Autoimmune Hemolytic Anemia as a Risk Factor of Poor Outcome in Patients with Splenic Marginal Zone Lymphoma

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Abstract Splenic marginal zone lymphoma is a rare disease, accounting for 1% of all lymphomas. We reviewed our single center experience of 13 patients with splenic marginal zone lymphoma (SMZL). Based on the prognostic model developed by Intergruppo Italiano Linfomi, 31% (4/13) of our patients had good, 38% (5/13) had intermediate and 31% (4/13) had a poor prognosis. The presence of two out of three prognostic factors (anemia, elevated LDH, low serum albumin) assignes the patient into the high risk category. In patients with anemia and an elevated LDH due to hemolysis, the outcome seems to be especially poor. Three out of 13 (23%) cases were complicated by autoimmune hemolytic anemia. All patients with autoimmune hemolytic anaemia (AIHA) died 7–28 months after the diagnosis. The mean follow-up time of those nine patients who are still alive is longer than 5 years (36–100 months). Patients with AIHA had significantly ($p<0.001$) worse survival than those without AIHA. The main finding of our study is that the presence of AIHA is an adverse prognostic factor in SMZL.

Keywords Autoimmune hemolytic anemia · Splenic marginal zone lymphoma · Prognosis · Outcome

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Introduction

In the current WHO classification, the term marginal zone lymphoma is used to identify three different entities: extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT), nodal marginal zone lymphoma (NMZL) and splenic marginal zone lymphoma (SMZL). Splenic marginal zone lymphoma is a rare disease, accounting for 1% of all lymphomas [1]. The term SMZL was used first by Schmid et al to characterize this special low-grade B-cell lymphoma of the spleen (1992) [2]. Splenic lymphoma with villous lymphocytes (SLVL) had been described a few years earlier [3]. Finally Isaacson et al reviewed the histological and immunophenotypical features of SLVL and concluded that SLVL and SMZL are one and the same entity [4]. The normal cellular counterpart of SMZL is currently unknown. The tumor cells are positive for the B-cell markers CD20 and CD79a, cytoplasmic monotypic immunoglobulin and Bcl-2 [1]. The disease affects elderly or middle aged patients in the sixth decade without gender predominance. The main disease features are splenomegaly, lymphocytosis and cytopenias often related to hypersplenism and, less frequently to autoantibodies. Lymphadenopathy, other organ involvement, B symptoms and increase of lactate dehydrogenase (LDH) are infrequent at presentation. The diagnosis of is based on a combination of features including lymphocyte morphology,

immunophenotype, cytogenetic abnormalities, BM histology and when available spleen histology. The diagnosis can even be made with the bone marrow biopsy, the intrasinusoidal infiltration is highly characteristic of SMZL. Immunohistochemical studies are extremely useful in the differential diagnosis [5]. Autoimmune phenomena are present in 9 to 20% of patients [6, 7]. SMZL has an indolent clinical course with a median survival of 10 years [6, 8], and a 5-year survival rate of 65–72% [9, 10]. The classical prognostic factors (International Prognostic Index, IPI) such as age, performance status, LDH level, stage or number of extranodal sites cannot prognostically distinguish among the SMZL patients [7]. Recently, Italian authors focused on detecting a subset of SMZL patients with unfavorable outcome and built a prognostic model for clinical use with the help of three readily available factors: anemia, LDH and serum albumin [6]. Moreover, it is of prognostic significance that in 10% of SMZL cases progression to a large B-cell lymphoma (LBCL) has been described. The transformation is mostly seen in the peripheral lymph nodes, it responds well to chemotherapy with durable progression-free survival. Transformation in the bone marrow is frequently refractory to therapy and is associated with poor outcome [11, 12]. Though the blastic transformation of low-grade B-cell lymphomas is well documented, the blastic transformation of SMZL is very rare [13]. The first patients with splenic lymphoma with villous lymphocytes complicated by autoimmune hemolytic anemia have been reported in 1992 [14].

Here we present our single-center experience with SMZL and report our observation regarding the prognostic significance of AIHA in SMZL.

Materials and Methods

Patients

Between May, 2000 and August, 2008, 17 patients were diagnosed with SMZL at the Division of Hematology, First Department of Medicine, Semmelweis University, Budapest, Hungary. In the present work we focus on those 13 patients, where long term (at least 3 years) follow-up data are available. Diagnoses were made based on the immunhistologic examination of bone marrow and/or spleen with the exception of the eldest patient.

The Following Data were Gathered demographic data, complete blood cell counts, serum LDH, serum albumin at the time of diagnosis, the presence of a serum monoclonal component, anti-HCV antibodies, HBsAg seropositivity, direct antiglobulin test (DAT), evidence of autoimmune disease, clinical symptoms, clinically detectable splenomegaly, lymphadenopathy, hepatomegaly, blood involvement: the presence of clonal (light chain-restricted) CD20+B lymphocytes and/or villous lymphocytes, bone marrow infiltration, Ann Arbor stage, prognosis according to the Italian prognostic model of SMZL (Arcaini et al) [6], type of treatment, response, response duration, follow-up, and causes of death.

Response Criteria

We used the following criteria to score and record clinical responses. A complete response (CR) was defined as the complete disappearance of all detectable sites of disease (including bone marrow involvement). Complete response undefined (CRu) was defined as a CR except that a bone marrow examination has not been performed. A partial response (PR) was defined as a reduction>50% for all measurable lesions. Progressive disease (PD) was considered an increase>25% in the size of previously documented disease, the appearance of disease at any site or a shift to a more aggressive histologic pattern.

Statistical Analysis

Statistical analysis was carried out using the SPSS 15.0 software. Continuous variables with normal distribution were presented with mean and standard deviation (SD) and variables with non-normal distribution were presented with median; minimum-maximum. Continuous variables were compared using Student's t-test or the Mann-Whitney U test and categorical variables were analyzed with the chi-square test. To assess AIHA and other variables associated with the outcome measures Kaplan-Meier survival plots were used with Log-Rank test.

Results

Clinical and pathological findings at the time of diagnosis are summarized in Tables 1 and 2. Response to therapy and outcomes are reported in Table 3. Among the 13 patients, there were 7 men and 6 women. The mean age was 67± 15 years (range 46–95). Ten patients showed bulky splenomegaly (> 10 cm below the left costal margin), 8 patients had minor hepatomegaly, and 4 patients had palpable lymphadenopathy. Four patients were found to have abdominal lymphadenopathy using abdominal ultrasound and/or CT. Unilateral hydrothorax was the consequence of pleural involvement (shown using cytology and flow cytometry) in one patient. A bone marrow trephine biopsy was performed in 12 patients. All but one had bone marrow involvement (of minor grade in 2 patients). Peripheral blood involvement was detected in 8 patients

Table 1 Main clinical data

Patients	Age (yrs)	Gender	Ann Arbor	Splenomegaly	LymphadenoP.	WBC (G/l)	ALC (G/l)	Hb (g/l)	LDH (U/l)	Prognosis*	Remarks	DLBCL**
1	95	M	-	Minor	-	21,5	17,84	127	308	Good	-	-
2	84	F	IV/B	Bulky	-	6,2	2,35	84	1469	Poor	AIHA	25 mo
3	82	F	II/A	Bulky	Abdominal	4,2	1,00	114	200	Intermediate	-	-
4	78	F	IV/A	Bulky	-	2,7	1,00	111	458	Intermediate	M:IgG λ	-
5	78	F	IV/B	Bulky	Abdominal	5,3	2,60	113	540	Poor	-	-
6	69	M	IV/A	Bulky	Abdom;mediast.	1,9	0,80	107	282	Intermediate	-	-
7	66	F	IV/A	Bulky	Spleen access.	20,0	17,40	109	424	Intermediate	AIHA	-
8	61	M	IV/A	Bulky	Peripheral	5,0	1,30	103	864	Poor	AIHA	-
9	59	M	III/A	Minor	Abdom;peripher.	5,9	1,00	134	259	Good	-	-
10	54	M	I/A	Bulky	-	5,1	2,14	137	277	Good	HCV,APS	-
11	52	M	IV/B	Bulky	Peripheral	13,7	10,96	86	804	Poor	M:IgM λ	96 mo
12	49	F	IV/B	normal	-	21,3	8,73	151	280	Good	-	-
13	46	M	IV/B	Bulky	Peripheral	16,0	10,88	120	643	Intermediate	HBV	20 mo

LympadenoP lymphadenopaties, *ALC* absolute lymphocyte count, *AIHA* autoimmune hemolytic anemia, *APS* antiphospholipid syndrome, *M* monoclonal component, *HCV* Hepatitis C Virus, Hepatitis B Virus

*according to Italian prognostic model (Arcaini et al.)

**after dg. of SMZL

Table 2 Pathological data

Patients	Diagnosis	Location of dg.	Confirm. dg. SMZL	BMI	Blood involvement	VL
1	SLVL	Blood	-	-	Yes	Yes
2	MZL	Bone marrow	Spleen	Moderate	Yes	No
3	SMZL	Bone marrow	-	Min	No	No
4	MZL	Bone marrow	Spleen	25–30%	No	No
5	SLVL	Bone marrow	Spleen	35%	Yes	Yes
6	SLVL	Bone marrow	-	10–15%	Yes	No
7	SMZL	Spleen		75%	Yes	Yes
8	SMZL	Spleen		n.e.	No	No
9	MZL	Lymph node	Spleen	0%	No	No
10	SMZL	Spleen		Min	No	No
11	SLVL	Blood	Spleen	35%	Yes	Yes
12	MZL	Blood	BMB, spleen	20%	Yes	Yes
13	MZL	Bone marrow	Spleen	45%	Yes	No

Cytogenetic aberrations, patient No.11: 47,XY,1q+, t(6,19),+19 [11], 47,X,-Y,1q+, t(6,19),+11,+19 [2]

BMB bone marrow biopsy, *BMI* bone marrow infiltration, *n.e.* not evaluable, *VL* villous lymphoytes

Table 3 Therapy and outcome

Patients	Th.1.	Response	Th.2.	Response	Th.3.	Response	Th.4.	Response	Th.5.	Response	Outcome	Months
1	Observation	SD									AWD	54
2	For AIHA	NR	SP	NR	For AIHA	Transient	R-CHOP				DD	28
3	Chlorambucil	PR	Rituximab								AWD	36
4	SP	HR									AW	51
5	SP	HR									AW	85
6	Fludarabine	PR									DOC	48
7	Sp.irradiation	PD	For AIHA	NR	SP	n.e.					DOC	9
8	For AIHA	NR	SP	NR	For AIHA	NR					DD	7
9	SP	PD	Fludarabine	CR							AW	76
10	SP	HR									AW	39
11	SP	HR	R-CHOP	CRu							AW	100
12	Observation	SyD	SP	SD							AWD	59
13	SP	PD	Fludarabine	n.e.	Orbitairradiation	LR	FC/FMC	NR	R-CHO	CRu	AW	68

SD stable disease, *SyD* symptomatic disease, *PD* progressive disease, *NR* no response, *n.e.* not evaluable, *PR* partial response, *LR* local response, *HR* hematological response, *CR* complete response, *CRu* unconfirmed CR, *SP* splenectomy, *FMC* Fludarabine-Mitoxantrone-Cyclophosphamide, *AW* alive and well, *AWD* alive with disease, *DD* dead of disease, *DOC* dead of other cause

(61.5%). Morphologically typical villous lymphocytes were found in 5 patients (38%). Because of the bone marrow involvement, 9 patients were Ann Arbor stage IV (69%). B symptoms were present at diagnosis in 5 patients (38%). The median hemoglobin level was 113 g/l (range 84–151) and the median platelet count was 141 G/l (range 79–294). The white blood cell count ranged from 1.9–21.5 G/l (median 5.3 G/l) and the median absolute lymphocyte count was 2.1 G/l (range 0.8–17.8). Lymphocyte count>5 G/l was seen in 5 patients (38%). No patient had<0.5 G/l neutrophil count. LDH levels were above normal in 5 patients (median 804, range 540–1469 U/l), secondary to hemolysis in 2 cases (864, 1469 U/l). All patients had a serum albumin level within the healthy reference range. Based on the prognostic model developed by Arcaini et al, 31% of our patients had good, 38% had intermediate and 31% had a poor prognosis [6]. A monoclonal component was detected in 2 patients (IgG λ; IgM λ). Hepatitis C virus (HCV) serology was positive in one patient, hepatitis B virus (HBV) surface antigen was detectable in another patient. This latter patient developed active HBV-related hepatitis during fludarabine therapy given for SMZL. One patient had evidence of antiphospholipid syndrome with a considerable prolongation (106 sec) of the activated partial thromboplastin time that normalized following splenectomy. In three out of 13 patients autoimmune hemolytic anemia was present already at the time of diagnosis. Two further patients were DAT positive serologically, but hemolytic anemia did not develop in either of them. None of the patients had an episode of ITP.

Patients with (n=3) vs without (n=10) AIHA had similar age (70±12 vs 66±17 years; p=NS). We also did not find any difference between these groups in serum hemoglobin (106±4 vs 120±18 g/l; p=NS); serum LDH (median; (min.-max.): 864; 424–1469 vs 295; 200–804 U/l; p=NS), white blood cells count (median; (min.-max.): 6.2; 5–20 vs 5.6; 1.9–21.5 G/l; p=NS), absolute lymphocyte cells count (median; (min.-max.): 2.35; 1.3–17.4 vs 2.37; 0.8–17.84 G/l; p=NS).

Response to Therapy and Outcome

One of the patients, the eldest one did not require treatment at all. Another patient was monitored using a wait-and-see policy until disease progression. Splenectomy (SP) was the first-line and only treatment in 6 and 3 patients, respectively. Four patients had SP later, in two of them the histological examination of the spleen led to the diagnosis of SMZL. One of these patients died some weeks following SP due to other cause (DOC). Chemotherapy alone was restricted to patients not fit for surgery. One patient received chlorambucil initially, resulting in 1 year of good PR following which progression was noticed and rituximab

treatment was initiated. One patient received fludarabine and obtained good PR that lasted until his death (DOC). Two patients received fludarabine therapy after splenectomy because of disease progression, one of them obtained CR. Three patients required combination chemotherapy (cyclophosphamide, daunorubicin, vincristine with or without prednisolone) because of high-grade transformation and two of them obtained CR. One very old patient died during the cytopenic period following chemotherapy (DD, dead of disease). One patient had repeated episodes of severe AIHA which ultimately proved to be refractory to high dose steroids, SP, cyclosporine, cyclophosphamide, repated cycles of IVIG and rituximab (DD).

Survival Analysis

Mean survival time for SMZL patients is 51 months, with a range from 7 to 100 months. Median survival was not reached at the moment of analysis, 9 patients (69%) were alive. The overall survival curve is shown in Fig. 1. In Fig. 2, the Kaplan-Meier plot was used to compare the patients' survival probability according to different prognostic groups. The survival plot of these sub-cohorts separate early and hold this tendency during the entire follow-up time. Males and females as well as patients with LDH in normal range vs out of normal range showed similar survival plots (not shown). We found similar results when comparing patients with vs without progression to DLBCL or non-splenectomized patients vs patients who underwent splenectomy. Patients with AIHA had significantly ($p<0.001$) worse survival than those without AIHA, as it is clearly demonstrated by the Kaplan-Meier plot (Fig. 3). All patients with AIHA and one out of ten patients without AIHA died during the follow-up period.

Discussion

The main finding of our study is that AIHA may be an adverse prognostic factor in SMZL. Observations on a frequent occurrence of immune disorders in SMZL/SLVL have been reported previously but in that evaluation a broad spectrum of associated autoimmune disorders have been included [7]. In our patients no episode of ITP was found, AIHA was the only immunologic disorder of prognostic significance. This is also important because in patients refractory to SP or not suitable for SP fludarabin has been regarded as secondline therapy. In view of our findings it is worthwhile to emphasize that rituximab is an effective treatment option in SMZL even as first line treatment with the additional safety of being a suitable treatment for AIHA as well. While rituximab is a treatment option in AIHA, fludarabin might exacerbate or provoke this potentially life-threatening condition. According to recent guidelines, patients with autoimmune hemolytic anemia (AIHA) or idiopathic thrombocytopenic purpura (ITP) should receive conventional treatments for these disorders prior to specific therapy for SMZL [5]. As second line treatment, rituximab might be of especial benefit in AIHA associated with SMZL.

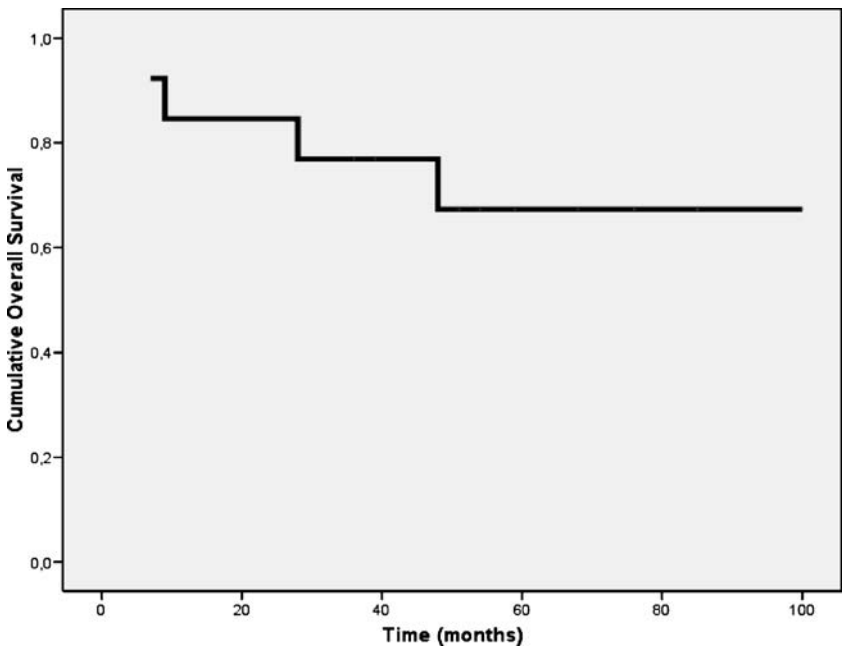

Fig. 1 Overall survival in our patients (Kaplan-Meier Plot)

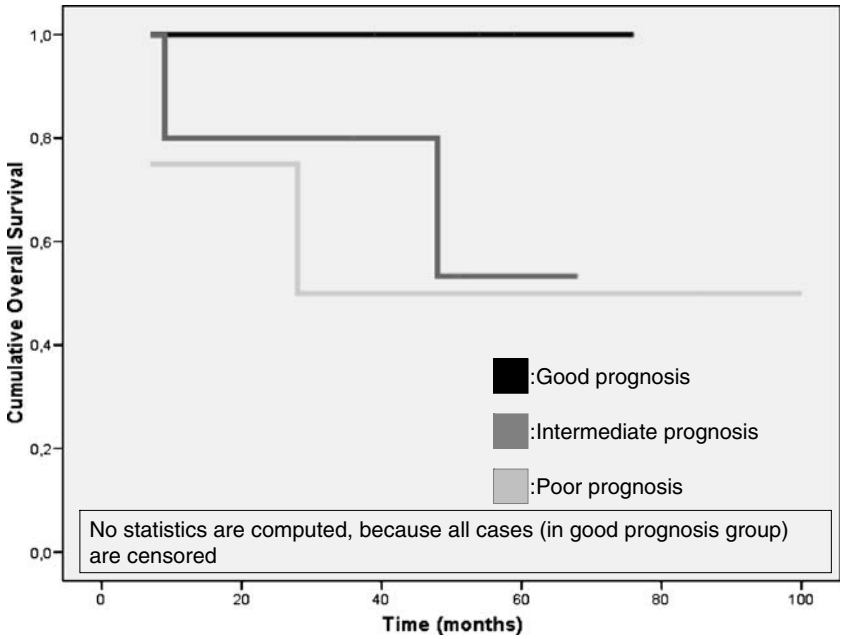

Fig. 2 Survival probability of SMZL patients according to the prognostic model developed by Arcaini et al (Kaplan-Meier Plot)

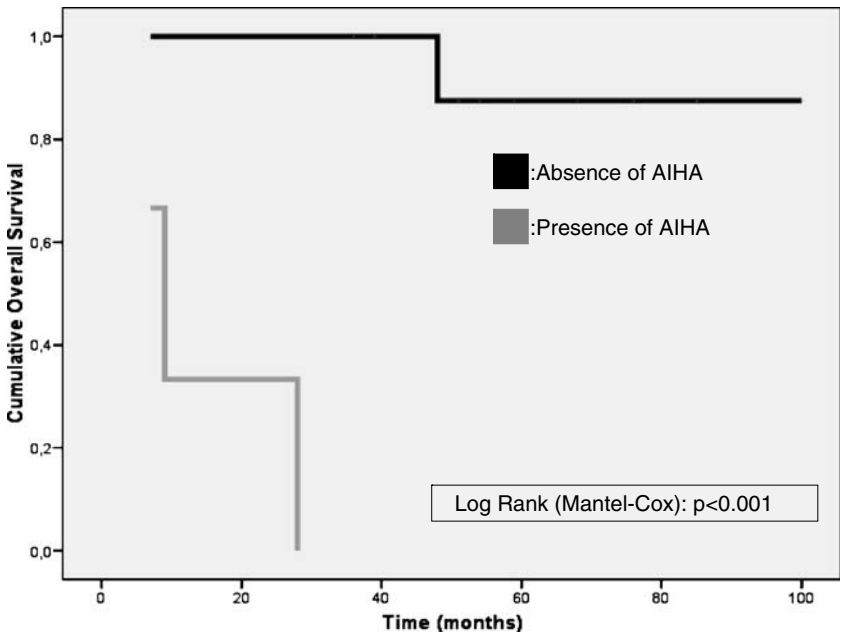

Fig. 3 Presence of autoimmune hemolytic anemia and survival (Kaplan-Meier Plot, Log Rank test: $p<0.001$)

Several limitations should be considered when interpreting our results. Our patients' number was small, consequently we are not able to perform correct statistical analysis to examine the independent association between AIHA and survival in patients with SMZL. On the other hand, the result of our study is good to create a new hypothesis whether the presence of AIHA is an independent and significant predictor of survival in patients with SMZL. Further studies including more patients and controlling some potential co-variables are needed to answer this question.

Conflict of interest None.

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