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Splenic marginal zone B-cell lymphomas: two cytogenetic subtypes, one with gain of 3q and the other with loss of 7q

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Background and Objectives. Splenic marginal zone B-cell lymphoma (SMZBCL) has clinical, immunophenotypic and histologic features distinct from other B-cell malignancies, but few chromosome studies have been previously reported. In the present study we performed conventional cytogenetics and *in situ* hybridization studies in 47 patients with SMZBCL.

Design and Methods. We studied 47 cases of splenic marginal zone B-cell lymphoma combining conventional cytogenetics and *in situ* hybridization (ISH) techniques using centromeric probes (chromosomes 3 and 12), locus specific probes (7q31 and 17p13) and cross-species color banding fluorescent ISH probes (RxFISH). The diagnosis of SMZBCL was ascertained in all cases after studying, morphologically and immunologically, peripheral blood and splenectomy specimens.

Results. A clonal chromosome abnormality detected by conventional cytogenetics and/or FISH was found in 33/47 patients (70%) being identified in 18 (18/33, 55%) as a complex abnormality. The most frequently recurrent abnormalities were: gain of 3q (10 cases), del(7q) (12 cases), and involvement of chromosomes 1, 8 and 14. No patient showed translocation t(11;14) (q13;q32) or t(14;18) (q21;q32). Trisomy 3 was detected in eight cases (8/47, 17%). Two novel cytogenetic abnormalities involving 14q32, t(6;14)(p12;q32) and t(10;14) (q24;q32) were reported. Deletion of 17p13 (P53) was observed by FISH in one case. Only one patient showed a gain of 3q or trisomy 3 and deletion 7q in the same karyotype.

Interpretation and Conclusions. Our findings support the interpretation that two forms of SMZBCL could be considered, one with gain of 3q and the other with deletions at 7q.

Key words: cytogenetics, *in situ* hybridization, splenic marginal zone B-cell lymphoma, splenic lymphoma with villous lymphocytes, cross-species color banding

Splenic marginal zone B-cell lymphoma (SMZBCL) is a recognized entity for which the clinical, morphologic, immunophenotypic and histologic characteristics are well established.[1-3] It is believed that SMZBCL has some overlapping features with splenic lymphomas with circulating villous lymphocytes (SLVL). SMZBCL may express circulating monocytoid B-cells, with or without microvilli and a variable number of lymphocytes very closely resembling those of SLVL. Bone marrow involvement in the absence of significant lymphadenopathy is also commonly seen at presentation.[4]

Although SMZBCL and SLVL appear to have clinical, immunophenotypic and histologic features distinct from other B-cell malignancies, chromosome studies have been previously reported only in a few series.[5-8] Oscier *et al.*[5] suggested a relationship with mantle cell lymphomas due to the detection of t(11;14), and Dierlamm *et al.*[6] found a high incidence of trisomy 3, similar to that reported in MALT lymphomas, in their series. In our previous work on 19 patients, we found a high incidence of involvement of chromosomes 1, 3, 7 and 8;[8] a lower frequency of trisomy 3 compared to the series of Dierlamm *et al.*[6] and no patient with t(11;14) (q13;q32).

The aim of the present study was to evaluate the cytogenetic data combining refined *in situ* hybridization techniques in a series of 47 patients with the diagnosis of SMZBCL based on histomorphologic and immunologic studies of spleen and peripheral blood.

Design and Methods

Patients

Forty-seven patients with a diagnosis of SMZBCL were studied (Table 1). The subjects were 47 consecu-

Table 1a. Cytogenetic findings in 47 patients with splenic marginal zone B-cell lymphoma (SMZBCL).

Num.	Conventional karyotype FISH	+3	+12	7q31	17p13
1.	46,XX [20].	N	N	N	N
2.*	48,XY,der(1), t(8;19)(?;q13),+mar(3), +mar [12]/ 46,XX [8].	+3	N	N	N
3.*	46,XX,del(6)(q21q24) [20].	+3	N	N	N
4.*	46,XY, ins(3;?)(p23;?) [20].	N	N	N	N
5.	46,XX,del(1)(q32) [7]/ 46,XX [13].	N	N	ND	ND
6.	46,XX,del(7)(q32) [20].	ND	ND	ND	ND
7.	46,XX [20].	N	N	ND	ND
8.	46,XX [20].	ND	ND	ND	ND
9.	46,XX [20].	N	N	DEL	N
10.	46,XX [20].	N	N	N	N
11.	46,XX [20].	N	N	N	N
12.	85-90, XXY, del(1)(q44), t(1;2)(q23;p21), del(3)(p11), der(4), del(5)(p13), del(6)(q23), del(9)(p21), dup(10)(q22q26), der(14), der(17), der(20) [20].	ND	ND	ND	ND
13.	44, XY, t(1;3)(q21;q22), t(7;17)(p11;p11), del(8)(q22),-20, -21 [20].	ND	ND	ND	ND
14.	46, XX, t(1;15)(p11;q11), del(8)(q12), del(18)(q21), del(14)(q24) [22]/46, XX [20]	ND	ND	ND	ND
15.	46, XX [20]	N	N	N	N
16*.	48, XX, +del(3)(p23), der(4)t(1;4)(q32;q35), +der(4)t(1;4)(q32;q35), add(14)(q32) [18]/ 46,XX [14].	+3	N	N	N
17.	46,XY, del(7)(q22), add(8)(q24), del(13)(q14) [22]/46,XY [17].	N	N	ND	ND
18*	46,XY,del(3)(q25),add(6)(q27),-7, del(13)(q14), del(14)(q11),-20,+mar(3), +mar [12]/ 46,XY[8].	+3	N	N	N
19	48,XY,del(3)(p23),add(8)(q24),add(13)(p11), add(15)(p11),+18, add(21)(p11),add(22)(p11), +mar(3) [18]/ 46,XY [12].	+3	N	ND	ND
20	46,XY,del(7)q21q31) [2]/46,XY [19]	N	N	ND	ND
21	46,XX,del(7)(q21q31) [3]/46,XX [17]	N	N	DEL	N
22	46,XY [12]	N	+12	N	N
23	45,X,-Y [5]/46,X,-Y,+3 [4]/46,XY [14]	+3	N	N	N
24	46,XX [23]	N	N	N	ND
25	46,XY, t(5;7)(p15;p11)) [8]/46,XY[12]	N	N	N	N
26*	49,XX, +5,+del(7)(q21), +der(7)t(3?;7) (p21;q22),i(8q) [8]/46,XX[6]	N	N	N	N
27	46,XX, del(7)(q31.3q34),der(16)t(12;16) (q13;q24) [6]/46,idem,,del(12)(p12p13) [1]/47,idem, +der(16) [1]/47,XX, +mar [2]/46,XX[19]	N	N	ND	ND

(continued in right column)

Table 1b. Cytogenetic findings in 47 patients with splenic marginal zone B-cell lymphoma (SMZBCL).

Num.	Conventional karyotype FISH	+3	+12	7q31	17p13
28	46,XY, del(1)(q42),+der(1), del(1)(p22),+3,+i(5)(q10),-9 [1]/ 48,idem, idic(9;15)(q10;q10)[1]/48,XY, del(1)(q11), +12,+15 [1]/46,XY[19]	+3	N	N	N
29	46,XX[10]	N	N	N	N
30	46,XY [20]	ND	ND	ND	ND
31	46,XY,del(7)(q32) [3]/45,XY,del(7)(q32), -22 [1] 46,XY,add(1)(q44), del(7)(q32) [1]/46,XY[1]	N	N	DEL	N
32	46,XX, del(1)(p32), del(3)(p13), der(8), der(9), del(11)(q21), der(17) [11]	+3	N	N	DEL
33	46,XX [20]	N	N	N	N
34	46,XX (19)/47,XX,+3 [1]	N	+12	N	N
35	46,XX [20]	N	N	N	ND
36*	46,XX,del(7)(q32q34) [15]	N	N	N	N
37	45,X,-Y [8]/45,XY,t(9;21)(q34;q21?),-19, [8]/ 45,XY,-20 [16]	N	N	N	N
38	46,XX, [20]	N	N	N	N
39	46,XX [20]	N	N	N	N
40	47,XY,+5 (2)/46,XY,der(5) [1]/ 47,XY,+1,der(5) [2]/ 47,XY, +5, del(7)(q32) [1]/ 46,XY [12]	N	N	N	ND
41*	46,XY,t(6;14)(p12;q32) [10] /46,XY [10]	N	N	N	N
42*	47,XY,der(6), del(7)(q32),der(19) t(11;19) (q11;q13),+mar [5]/46,XY,der(6), add(18)(q23) [4]/ 46,XY,der(6), del(7)(q32) [4]/ 46,XY [3]	N	N	N	N
43	46,XX [20]	N	N	N	N
44*	46,XX, del(3)(p21), +del(3)(p21), +17, der(21)t(17;21)(q11;q22) [4]/46,XX [16]	+3	N	N	N
45*	46,XX,t(8;22)(q22;q13) [15]/46,XX [5]	N	N	N	N
46	47,XX,+X, der(4) t(4;17)(p16;q11), del(7)(q32), t(6;14)(p12;q32), t(8;15) (q12;q26) [15]/46,XX [22]	N	N	ND	ND
47*	46,XY,del(7)(q22),add(12)(p13)[10]/ 46,XY[10]	N	N	DEL	N

**Patients in whom RxFISH was applied to define the karyotype. mar(3). Marker with material from chromosome 3 (found by the painting method). FISH: fluorescence in situ hybridization. DEL: deletion. ND: not done. N: normal by FISH.*

tive patients who were referred to hospitals belonging to the Spanish Cytogenetic Group between 1994 and 1999. In the present series we included only patients in whom the diagnosis was histologically confirmed by the authors; all the patients were T-cell marker negative and expressed B-cell markers. The patients were included according to the criteria of Mollejo *et al.*[2] The diagnosis of SMZBCL was made in all cases after study of the

splenectomy specimens. No cyclin-D1 overexpression was detectable in any of the tissue from splenectomy specimens. Circulating villous lymphocytes were observed in 63% of the patients.

Cytogenetic and some clinical information concerning the first 19 patients and patients with t(6;14)(p12;q32) (cases #41 and 46) have been previously reported.[8,9]

Methods

Cytogenetic studies. The cytogenetic studies were performed in all patients at diagnosis prior to any treatment or at the moment of the splenectomy. Chromosome analysis was carried out on lymphoid cells from peripheral blood, lymph nodes or from spleen. The following mitogens were used: phytohemagglutinin (PHA) and phorbol-myristate-acetate (TPA). Cultures were incubated for 72 hours at 37ºC. Cytogenetic analysis was performed at the individual hospitals. The results were reviewed and collected centrally by the main investigator (*FS*). G-banding was performed after having treated the preparations in a slide warmer at 100ºC for one hour, and then stained them with Wright's solution. A minimum of 20 metaphases were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).[10]

Interphase in situ hybridization. Fluorescent *in situ* hybridization (FISH) was performed with chromosomes 3 and 12-specific α-satellite DNA probes (CEP3 and CEP12, Vysis, Downers Grove, USA). For locus specific probes we used 7q31 and 17p13 (P53) (LSI 7q31 and LSI P53, Vysis, Downers Grove, USA). A minimum of 500 nuclei and 200 nuclei, respectively, were analyzed for centrometric probes and locus-specific probes. Cut-offs were defined by adding three standard deviations to the mean of the frequency of blood cells with +3, +12, del(7)(q31) or del(17)(p13) in ten normal specimens. We considered that there was a gain of a chromosome when the percentage of cells with trisomy was more than 5% and a loss of chromosome when more than 10% of cells presented the anomaly. FISH studies were studied centrally by the main investigators (*FS*, *MS* and *BE*).

Cross-species color banding (RxFISH). RxFISH was applied in some patients from whom material was available. RxFISH was performed according to the procedures supplied by the manufacturer. Briefly, metaphase slides were prepared the same day and aged for 1 hour in a 100ºC slidewarmer. Ten microliters of RxFISH color chromosome probe (RxFISH kit, Applied Imaging, Santa Clara, CA, USA) per case were pre-warmed for 5 minutes at 37ºC, then denatured at 65ºC for 10 minutes and placed in a 37ºC waterbath for at least 10 minutes (used within two hours). Preparations were dehydrated in 100% ethanol at room temperature for 5 minutes and allowed to dry naturally. They were then denatured by incubation in a 70% formamide/2XSSC solution for 1.5 minutes at 72ºC. Slides were immediately quenched in ice cold 70% ethanol for 2 minutes and dehydrated using 70%, 90% (twice) and 100% ethanol series for 2 minutes each at room temperature. Slides were allowed to dry naturally. Ten microliters of the denatured RxFISH probes were applied to each slide, which was covered by a coverslip. Slides were incubated for at least 12 hours in a moist chamber at 37ºC. Post-hybridization washes consisted of one change of five minutes in a 2XSSC solution, two changes of five minutes each in a 50% formamide/2XSSC solution, one wash of five minutes in a 2XSSC solution and one wash of ten minutes in a 4xSSC/0.05% Tween-20 solution at 45ºC. Slides were stained in a Coplin jar containing DAPI (4,6-diamino-2-phenylindole)/2XSSC (0.1 µg/mL) for at least two minutes at room temperature. Finally, they were mounted on Citifluor AF1 mounting media and covered by a coverslip. Results were analyzed in a fluorescent Nikon (Eclipse 600) microscope using the Cytovision software to analyze RxFISH. A minimum of 20 metaphases per case were studied by two different observers.

Results

Mitoses were obtained in all patients. Out of 47 patients, 33 (70%) showed clonal karyotypic abnormalities (Table 1). One patient showed trisomy 3 in a single metaphase but clonality was not confirmed by FISH.

Cytogenetic results are summarized in Tables 1, 2 and 3. The chromosomal breakpoints found in at least two patients were: 1p22, 1p32, 1q32, 1p42, 3p13, 3p21, 3p23, 7q21→7q34, 8q24, 13q14, 14q32 and 19q13. A translocation t(6;14)(p12;q32) was observed in two patients, in one of them as a sole anomaly, and in the other as a complex karyotype including a deletion at 7q32. The most frequent structural aberration was deletion 7q (12 patients). In two patients a rearrangement of 8q24 was found, in one as a translocation t(8;22)(q24;q11) and in the other as an add(8)(q24). A deletion del (13)(q14) was detected in two patients, in both as a complex karyotype. With respect to numerical abnormalities, a gain of material from chromosome 3 was observed in 10 patients, trisomy 5 in three, trisomy 12 in two, monosomy 20 in three and loss of Y chromosome in two.

One patient had deletion 7q and gain of 3q in the same karyotype. Regarding these aberrations, gain of 3q was detected in two patients as a single abnormality and in 8 as part of a complex karyotype. Deletion 7q was found in four patients as a sole anomaly, in one with a normal karyotype it was detected by FISH and in seven it was part of a complex karyotype.

Eighteen out of 33 patients had a complex karyotype (more than three chromosomes involved). In patients with a complex karyotype, the application of RxFISH enabled the identification of novel cytogenetic abnormalities (Table 4): t(1;15)(p36;q?), t(3;17)(q25;q21), t(3;18)(p11;q11), del(5)(q22), t(7;10)(q22;q24), t(6;14)(q27;q13), i(9)(q10), t(10;14)(q24;q32), del(15)(q22) and t(X;15)(q27;?). No patient showed a t(11;14), a t(14;18), or translocations of 3q27.

Comparing FISH with conventional cytogenetic findings, trisomy 3 was detected in nine cases by FISH and

Table 2. Frequency of chromosomes implicated in cytogenetic abnormalities in 47 patients with splenic marginal zone B-cell lymphoma (SMZBCL).

Chromosomes involved	*Frequency (%)*
1	10/33 (30%)
2	2/33 (6%)
3	17/33 (51%)
4	3/33 (9%)
5	6/33 (18%)
6	6/33 (18%)
7	17/33 (51%)
8	7/33 (21%)
9	4/33 (12%)
10	0/33
11	2/33 (6%)
12	5/33 (15%)
13	3/33 (9%)
14	8/33 (24%)
15	4/33 (12%)
16	0/33
17	5/33 (15%)
18	3/33 (9%)
19	3/33 (9%)
20	4/33 (12%)
21	3/33 (9%)
22	2/33 (6%)
X	3/33 (9%)
Y	2/33 (6%)

Table 3. Numerical and structural abnormalities detected in a series of 47 patients with SMZBCL.

Numerical *Chromosome*	*Incidence*
+1	1/33
+3	10/33
+5	3/33
-7	1/33
-9	1/33
+12	2/33
-20	3/33
-Y	2/33
Structural *Chromosome*	*Incidence*
1p- (1p22, 1p32)	2/33
1q- (1q32, 1q42)	4/33
3p- (3p13, 3p21, 3p23)	6/33
+3q	11/33
7p- (7p11)	2/33
7q- (7q21Æ7q34)	12/33
t(6;14)(p12;q32)	2/33
involvement of 8q24	2/33
del(13q14)	2/33
involvement of 14q32	3/33
der(17)	5/33
involvement of 19q13	3/33
involvement of #21	3/33

in seven by cytogenetics; trisomy 12 in one by FISH and in one by cytogenetics, deletion 7q in four by FISH (one patient with a normal karyotype, one with a deletion at 7q21, one with a deletion at 7q22 and one with a deletion at 7q32) and in 10 by conventional cytogenetics; and deletion 17p13 was detected in one patient by FISH (Table 5).

Regarding the prognostic value of karyotype, nine patients died after the following periods of survival: case #3 (42 months), case #6 (45 months), case #7 (9 months), case #12 (17 months), #13 (57 months), #20 (24 months), #23 (2 months), #24 (49 months) and #47 (23 months). Of these patients, three had a complex karyotype (mean survival time, 32 months), three had trisomy 3 (mean survival time, 20 months) and three had a 7q deletion (mean survival time, 31 months).

Discussion

The present study deals with the cytogenetic findings in a group of 47 histologically proven cases of SMZBCL. The overall incidence of clonal chromosome abnormalities in our series was 70%. Oscier *et al.*[5] found cytogenetic aberrations in 27/31 patients with SLVL (87%), and Dierlamm *et al.*[7] reported 23 patients (74%) with an abnormal karyotype in a series of 31 cases of SMZBCL . In a series of 76 cases, Troussard *et al.*[11] observed that 19 (43%) presented cytogenetic aberrations, predominantly t(11;14)(q13;q32) (5 cases), involvement of chromosome 3 and trisomy 12. The different incidences of chromosomal abnormalities could be due to the small size of the series and to different inclusion criteria, because previous reports were based only on cytologic and immunologic study of peripheral blood smears.

In our present series, the chromosomes most frequently involved were 1, 3, 7, 8, 13, 14 and 20; the finding of deletions 1q32, 3p23 and of 7q (7q22 and 7q32) is particularly interesting. Regarding the presence of translocation t(11;14)(q13;q32), we were not able to detect this translocation in any of our 47 patients, whereas Oscier *et al.*[5] found it in 5 out of 31 cases, and Troussard *et al.*[11] in 5 out of 76 cases. Additional findings in the series of Oscier *et al.*[5] were deletions and translocations involving 7q, iso17q and 2p11. In the series studied by Dierlamm *et al.*[7] no patient showed either translocation t(11;14) or rearrangements of the BCL-1, BCL-2, BCL-3, BCL-6 and c-MYC genes. The most frequent clonal abnormalities of their series included whole or partial trisomy 3 (18 patients), trisomy 18 (9 patients) and structural rearrangements of chromosome 1 (15 patients) with breakpoints in 1q21 or 1p34. We found trisomy 3 in 10 of out 33 patients (incidence 30%). Dierlamm *et al.*[6] found an incidence of trisomy 3 of 55% (6/11) in splenic marginal zone B-cell lymphoma (MZBCL), of 67% (8/12) in extranodal MZBCL and of 62% (8/13) in nodal MZBCL. In the series examined by Oscier *et al.*[5] only one patient among 27 had a derivative chromosome 3. Wotherspoon *et al.*[12] found an incidence of trisomy 3 of 60% in 70 patients with MALT lymphomas. Brynes *et al.*,[13] in a retrospective study of 36

Table 4. Comparative cytogenetic abnormalities detected by conventional cytogenetics and RxFISH.

CASE	*Conventional cytogenetic findings*	*RxFISH findings*
2	48,XY, der(1), t(8;19)(?;q13),+mar(3), +mar [12]	48,XY,der(1)t(1;15)(p36;q?),+der(3)t(3;17)(q25;q21), der(20)t(8;20) (q?;q13) [4]
3	46,XX,del(6)(q21q24) (4)/46,XX [16]	46,XX [20]
4	46,XY, ins(3;?)(p23;?) [20]	46,XY [20]
16	48,XX, +del(3)(p23), der(4)t(1;4)(q32;q35), +der(4)t(1;4)(q32;q35), add (14)(q32)	48 X,der(X),+der(X), +3, del(8)(p11), t(10;14)(q24;q32), 9qh+ [20]
18	46,XY, del(3)(q25), add(6)(q17),-7,del(13)(q14), del(14)(q11), -20, +mar 3, +mar [12]	46,XY, del(3)(p21),+der(3),- 5, t(6;14)(q27;q13), del(13)(q14),+del(15)(q22) 46,XY, del(3)(p21),+der(3),-5, del(5)(q22), t(6;14) (q27;q13), del(13)(q14)
26	50,XX,+5,del(7q),+der(7)t(3;7)(p21;q22), i(8q) [8] 46,XX [6]	°79-99, XX,+der(X)t(X;15)(q27;?),+der(X)t(X;15)(q27;?), +1,+2+2,+3,+3,+4,+5,+5, +7,+der(7)t(7;10)(q22;q24), +der(7)t(7;10)(q22;q24),+der(7)t(7;10)(q22;q24), +der(7)t(7;10)(q22;q24),+i(9q),+i(9q),+i(9q),+10,+10,+11, +11,+12,+13, +14, +15,+16,+16,+18,+18, +19,+20 [8] 46,XX [12]
36	46,XX, del(7)(q32q34) [20]	46,XX [4] 46,XX, del(7)(q32q34)[16]
41	46,XY,t(6;14)(p12;q32) [10]/ 46,XY [10]	46,XY,t(6;14)(p12;q32) [10]/ 46,XY [10]
42	47,XY,der(6), del(7)(q32), der(19)t(11;19) (q11;q13), +mar [5]/46,XY, der(6), add(18)(q23) [4]/ 46,XY, der(6), del(7)(q32) [4]	47,XY,der(6)t(3;6) [10] Poor quality
44	47,XX, del(3)(p21), +del(3)(p21), +17, der(21)t(17;21)(q11;q22) [6]	47,XX,del(3)(p12),+der(3)t(3;18)(p11;q11),+der(3)t(3;18)(p11;q11),-18 [10]
45	46,XX [5] 46,XX, t(8;22)(q24;q11) [15]	46,XX,t(8;22)(q24;q11) [20]
47	46,XY[10]/46,XY,del(7)(q22),add(12)(p13)[10]	46,XY[10]/46,XY,del(7)(q22), der(12)t(3;12)(q21;p13)[10]

°Disease progression.

cases of MZBCL studied by fluorescence *in situ* hybridization, identified trisomy 3 in 11 (85%) extranodal MZBCL with monocytoid B cells, in 6 (50%) of 12 nodal MZBCL of monocytoid B-cell type, and in only 2 (18%) SMZBCL. Recently Gruszka-Westwood *et al.*[14] analyzed, by FISH, a series of 70 patients with the diagnosis of SLVL and found an incidence of trisomy 3 of 17%. In our first report[8] and in the present one, the incidence of trisomy 3 in SMZBCL patients is lower than that observed in the remaining marginal lymphomas.

In our previous series of SMZBCL, we found deletion 7q (7q22 and 7q32) in two patients; the incidence of this abnormality is higher in the present study (35%). Concerning the use of the commercial probe (LSI 7q31) to detect this anomaly, conventional cytogenetics studies are preferable because they can detect terminal deletions of 7q. Deletion del(7)(q32) is found in some low-grade lymphomas with circulating lymphoid cells showing plasmacytoid features.[15] We were first in presenting this cytogenetic aberration in four patients with B-cell chronic lymphoid disorders, two of them with the diagnosis of SLVL.[16] Frequently such patients share multiple karyotypic abnormalities other than del(7q). This finding points to a secondary progression-related role for a tumor-supressor gene at 7q32. Nevetheless, in our series four patients showed deletions at 7q (7q32, 7q21q31 in two cases and 7q32q34) as a sole abnormality, suggesting a pathogenetic role. Mateo *et al.*,[17] in a series of 20 patients with the diagnosis of SMZBCL, studied by means of 7q LOH, found 40% of patients with deletion at 7q, and this group of patients had higher tumoral progression. Recently, Corcoran *et al.*[18] studied four patients with a diagnosis of SLVL, and found a translocation involving 7q21-22. It is interesting to note that the CDK6 gene is mapped in 7q22, and that in two patients overexpression of the CDK6 protein was detected. The same authors investigated[15] additional cases of SLVL without abnormalities of chromosome 7q and failed to demonstrate genomic rearrangements of CDK6.

These results suggested that dysregulation of CDK6 gene expression could contribute to the pathogenesis of SLVL and SMZBCL. According to this work we supposed that some of our cases might have a translocation of 7q and not a deletion. However, using the the RxFISH probe and locus specific probe from 7q31, none of our patients with a previous del(7q) had a translocation of this

Table 5. Cytogenetic and FISH results in a series of 47 SMZBCL patients.

Anomaly	*Conventional cytogenetics*	*FISH*
Trisomy 3	7/47 (15%)	9/47 (19%)
Trisomy 12	1/47 (2%)	1/47 (2%)
Deletion 7q	12/47 (25%)	4/47 (8.5%)
Deletion 17p13	0/47	1/47 (2%)

region, although a translocation t(7;10)(q22;q24) was detected in one case (case #26). This translocation was not reported by other authors.[19] Taking into account our results we consider that translocations of 7q are infrequent in SMZBCL and that the most frequent anomaly is a deletion at 7q. Recently, Knuutila's group summarized reports of recurrent DNA sequence copy number (losses and gains) in human neoplasms as detected by comparative genomic hybridization, and revealed deletion 7q as a recurrent loss in lymphomas.[20,21]

It is worth mentioning that only one patient with a deletion of 7q presented a gain of 3q. To our knowledge, patients with gain of 3q do not have deletion at 7q.[5,15,7,11] It is interesting to study the clinical and histologic data of both groups of patients. Trisomy 3 was frequently observed in patients with complex karyotypes whereas deletion 7q was found in patients as a single anomaly. Taking into account the rare coexistence of both abnormalities, we can argue that two different pathways could be established: one related to the amplification of 3q and the other related to a deletion at 7q.

New rearrangements of 14q32 were observed: two cases with t(6;14)(p12;q32) and one case with t(10;14)(q24;q32). A new translocation t(6;14)(p12;q32) was observed in two previously reported cases.[9] In one of them (case #41) it was the sole anomaly and in the other (case #46), a complex karyotype with deletion 7q32 was observed. Interestingly, case #36 had a deletion of 7q32 at diagnosis and during the evolution of the disease acquired a complex translocation t(6;8;14)(p12;q24,q32), suggesting that t(6;14) could be related to progression of the disease. Translocation t(10;14)(q24;q32) has not been previously reported.[19] It is interesting to note that in our present series a case with a translocation involving 10q24 was observed.

The recent development of a cross-species color banding technique (RxFISH) has permitted refinement of karyotype analysis allowing the detection of chromosomal rearrangements that involve the same chromosome, such as translocations among both homologs, inversions, or insertions between homologs.[22-26] In our patients with complex karyotypes, this technique allowed the identification of previously unrecognized cytogenetic aberrations and allowed the karyotype to be accurately defined. In the present series, RxFISH identified the following novel aberrations: t(1;15)(p36;q?), t(3;17)(q25;q21), t(3;18)(p11;q11), del(5)(q22), t(7;10)(q22;q24), t(6;14)(q27;q13), i(9)(q10), t(10;14)(q24;q32), del(15)(q22) and t(X;15)(q27;?).

There are no reported data about the prognosis of cytogenetic aberrations in SMZBCL. Although the follow-up of our patients is short, nine patients have so far died, and of them, three had a complex karyotype (mean survival time, 32 months), three had trisomy 3 (mean survival time, 20 months) and three had a deletion 7q (mean survival time, 31 months). We consider that a very large co-operative study should be undertaken to clarify the prognostic significance of the cytogenetic findings in patients with SMZBCL.

To conclude, molecular cloning of 3q and 7q breakpoints may define new DNA sequences of oncogenic potential at these loci. Further studies are needed to understand the significance of 3q and 7q and the new cytogenetic aberrations detected by RxFISH in SMZBCL, in order to reach a better evaluation of histologic and clinical correlations.

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FS collected the data and wrote the manuscript. FS coordinated the entire project. FS reviewed the cytogenetic findings. MS was involved in collection of the clinical data of all the patients. MS and BE were responsible for the in situ *hybridization studies. JH, JAMC, IG, JLG performed the cytogenetic studies. LF, SW, TV, AD, IB reviewed the morphology of the patients. MM, MAP and SS reviewed the splenectomy specimens. All the authors gave their critical contribution to the manuscript and approved its final version. We would like to thank Rosa Maria Vila and Rosa Navarro for their expert technical assistance.*

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Potential implications for clinical practice

The accurate diagnosis of patients with SMZBCL, taking into account cytogenetic data, will have very useful therapeutic implications.

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